

WHAT IS CLAIMED IS:

1. A method of producing human growth hormone in a duckweed plant culture or a duckweed nodule culture, comprising the steps of:
- 5 (a) culturing within a duckweed culture medium a duckweed plant culture or a duckweed nodule culture, wherein said duckweed plant culture or said duckweed nodule culture is stably transformed to express said human growth hormone, and wherein said human growth hormone is expressed from a nucleotide sequence comprising a coding sequence for the human growth hormone and an operably linked
- 10 coding sequence for a signal peptide that directs secretion of the human growth hormone into the culture medium; and
- (b) collecting said human growth hormone from the duckweed culture medium.
- 15 2. The method of claim 1, wherein said human growth hormone is secreted into the duckweed culture medium.
3. The method of claim 1, wherein said nucleotide sequence has at least one attribute selected from the group consisting of:
- 20 (a) duckweed-preferred codons in the coding sequence for said human growth hormone;
- (b) duckweed-preferred codons in the coding sequence for said signal peptide;
- (c) a translation initiation codon that is flanked by a plant-preferred translation initiation context nucleotide sequence; and
- 25 (d) an operably linked nucleotide sequence comprising a plant intron that is inserted upstream of the coding sequence; and
- (e) an operably linked nucleotide sequence comprising the leader sequence from the ribulose-bis-phosphate carboxylase small subunit 5B gene of
- 30 *Lemna gibba*.

4. The method according to claim 3, wherein said duckweed-preferred codons are *Lemna gibba*-preferred codons or *Lemna minor*-preferred codons..

5 5. The method according to claim 4, wherein at least one coding sequence selected from the coding sequence for said polypeptide and the coding sequence for said signal peptide comprises between 70-100 % *Lemna gibba*-preferred codons or *Lemna minor*-preferred codons..

10 6. The method according to claim 3, wherein said plant-preferred translation initiation context nucleotide sequence consists of the nucleotide sequence "ACC" or "ACA", wherein said context is positioned immediately adjacent to of the 5' end of the translation initiation codon.

15 7. The method according to claim 3, wherein said operably linked nucleotide sequence comprising said plant intron is the sequence set forth in SEQ ID NO:1.

8. A method of producing an antibody in a duckweed plant culture or a duckweed nodule culture, comprising the steps of:

- 20 (a) culturing within a duckweed culture medium a duckweed plant culture or a duckweed nodule culture, wherein said duckweed plant culture or said duckweed nodule culture is stably transformed to express said antibody, and wherein said antibody is expressed from one or more nucleotide sequences comprising a coding sequence for a chain of the antibody and an operably linked coding sequence for a signal
- 25 peptide that directs secretion of the antibody into the culture medium; and
- (b) collecting said antibody from the duckweed culture.

9. The method of claim 8, wherein said one or more nucleotide sequences have at least one attribute selected from the group consisting of:

- (a) duckweed-preferred codons in the coding sequence for the chain of the antibody;
- 5 (b) duckweed-preferred codons in the coding sequence for said signal peptide;
- (c) a translation initiation codon that is flanked by a plant-preferred translation initiation context nucleotide sequence;
- (d) an operably linked nucleotide sequence comprising a plant intron
10 that is inserted upstream of the coding sequence; and
- (e) an operably linked nucleotide sequence comprising the leader sequence from the ribulose-bis-phosphate carboxylase small subunit 5B gene of *Lemna gibba*.

15 10. The method according to claim 9, wherein said duckweed-preferred codons are *Lemna gibba*-preferred codons or *Lemna minor*-preferred codons.

11. The method according to claim 10, wherein the coding sequence comprises between 70% and 100% *Lemna gibba*-preferred codons or *Lemna minor*-
20 preferred codons.

12. The method according to claim 9, wherein said plant-preferred translation initiation context nucleotide sequence consists of the nucleotide sequence "ACC" or "ACA", wherein said context is positioned immediately adjacent to the 5' end of the
25 translation initiation codon.

13. The method according to claim 9, wherein said operably linked nucleotide sequence comprising said plant intron is the sequence set forth in SEQ ID NO:1.

14. The method according to claim 8, wherein said duckweed frond culture or duckweed nodule culture expresses and assembles the heavy chain and light chain of the antibody.

5 15. The method according to claim 8, wherein said antibody is a Fab' fragment.

16. The method according to claim 8, wherein said antibody is a mAb

10 17. The method according to claim 8, wherein the antibody is a human antibody.

15 18. The method according to claim 1, wherein said human growth hormone has at least 90% sequence identity with the amino acid sequence set forth in SEQ ID NO: 15.

19. The method according to claim 18, wherein the human growth hormone has the amino acid sequence set forth in SEQ ID NO:15.

20 20. The method according to claim 1, wherein the coding sequence for the human growth hormone comprises the nucleotide sequence set forth in SEQ ID NO:14.

25 21. The method according to claim 1, wherein said signal peptide sequence has the amino acid sequence set forth in SEQ ID NO:13.

22. The method according to claim 21, wherein the coding sequence for the signal peptide comprises the nucleotide sequence set forth in SEQ ID NO:14.

30 23. The method according to claim 8, wherein said signal peptide sequence has the sequence set forth in SEQ ID NO:6.

24. The method according to claim 23, wherein the coding sequence for the signal peptide comprises the nucleotide sequence set forth in SEQ ID NO:3.

5 25. The stably transformed duckweed plant culture or duckweed nodule culture according to claim 1.

26. The stably transformed duckweed plant culture or duckweed nodule culture according to claim 25, wherein said duckweed plant culture or duckweed nodule
10 culture is selected from the group consisting of the genus *Spirodela*, genus *Wolffia*, genus *Wolffiella*, and genus *Lemna*.

27. The stably transformed duckweed plant culture or duckweed nodule culture according to claim 26, wherein said duckweed plant culture or duckweed nodule
15 culture is selected from the group consisting of *Lemna minor*, *Lemna miniscula*, *Lemna aequinoctialis*, and *Lemna gibba*.

28. The stably transformed duckweed plant culture or duckweed nodule culture according to claim 8.

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29. An isolated nucleic acid molecule comprising a nucleotide sequence encoding an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence set forth in SEQ ID NO:15;
- (b) the amino acid sequence of a variant of the human growth
25 hormone sequence shown in SEQ ID NO:15, wherein said variant has at least about 90% sequence identity with the amino acid sequence set forth in SEQ ID NO:15; and

wherein said nucleotide sequence comprises duckweed-preferred codons.

30. A nucleic acid molecule comprising a nucleotide sequence encoding the human growth hormone signal peptide amino acid sequence set forth in SEQ ID NO:13; wherein said nucleotide sequence comprises duckweed-preferred codons.

5 31. A method of producing human α -interferon in a duckweed plant culture or a duckweed nodule culture, comprising the steps of:

(a) culturing within a duckweed culture medium a duckweed plant culture or a duckweed nodule culture, wherein said duckweed plant culture or said duckweed nodule culture is stably transformed to express said human α -interferon, and
10 wherein said α -interferon is expressed from a nucleotide sequence comprising the leader sequence from the ribulose-bis-phosphate carboxylase small subunit 5B gene of *Lemna gibba* operably linked to a coding sequence for the human growth hormone, and an operably linked coding sequence for a signal peptide that directs secretion of the α -interferon into the culture medium; and

15 (b) collecting said α -interferon from the duckweed culture medium.

32. The method of claim 31, wherein said α -interferon is secreted into the duckweed culture medium.

20 33. The method of claim 31, wherein said nucleotide sequence has at least one attribute selected from the group consisting of:

(a) duckweed-preferred codons in the coding sequence for said α -interferon;

(b) duckweed-preferred codons in the coding sequence for said signal peptide;
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(c) a translation initiation codon that is flanked by a plant-preferred translation initiation context nucleotide sequence; and

(d) an operably linked nucleotide sequence comprising a plant intron that is inserted upstream of the coding sequence.
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34. The method according to claim 33, wherein said duckweed-preferred codons are *Lemna gibba*-preferred codons or *Lemna minor*-preferred codons..

35. The method according to claim 34, wherein at least one coding sequence
5 selected from the coding sequence for said polypeptide and the coding sequence for said signal peptide comprises between 70-100 % *Lemna gibba*-preferred codons or *Lemna minor*-preferred codons..

36. The method according to claim 33, wherein said plant-preferred
10 translation initiation context nucleotide sequence consists of the nucleotide sequence "ACC" or "ACA", wherein said context is positioned immediately adjacent to of the 5' end of the translation initiation codon.

37. The method according to claim 33, wherein said operably linked
15 nucleotide sequence comprising said plant intron is the sequence set forth in SEQ ID NO:1.

38. The method of claim 31, wherein the leader sequence from the ribulose-
bis-phosphate carboxylase small subunit 5B gene of *Lemna gibba* is the nucleotide
20 sequence shown in SEQ ID NO:16.

39. The method of claim 31, wherein the signal peptide has the amino acid sequence set forth in SEQ ID NO:6.

25 40. The stably transformed duckweed plant culture or duckweed nodule culture according to claim 31.

41. Human growth hormone produced according to the method of claim 1.

30 42. An antibody produced according to the method of claim 8

43. α -interferon produced according to the method of claim 31.

44. A method of enhancing the expression of a biologically active polypeptide
5 in duckweed, said method comprising culturing a duckweed plant culture or a duckweed
nodule culture, wherein said duckweed plant culture or said duckweed nodule culture is
stably transformed to express said biologically active polypeptide and wherein said
biologically active polypeptide is expressed from a nucleotide sequence comprising a
coding sequence for the biologically active polypeptide and an operably linked nucleotide
10 sequence comprising the leader from the ribulose-bis-phosphate carboxylase small
subunit gene of *Lemna gibba*.

45. The method of claim 44, wherein said leader from the ribulose-bis-
phosphate carboxylase small subunit gene of *Lemna gibba* has the nucleotide sequence
15 set forth in SEQ ID NO:16.